Cellular kinases modulate KSHV latency maintenance

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Abstract Developing specific therapies for Kaposi's Sarcoma (KS) is challenging as few virus-specific targets exist in latently infected KS tumors. Therefore, identifying effective pharmacological agents for KS treatment could be enhanced by a better understanding of cellular mediators of KSHV latency. Kinases are important regulatory proteins that control many host and viral processes, including oncogenesis. For this reason, kinase inhibitors are among the most successful targeted cancer therapies, with >50 FDA-approved drugs and hundreds in clinical development. Elucidating kinase-dependent signaling and pharmacological agents effective in regulating KSHV latency maintenance would provide critical insights into KSHV latency dependency factors and potential therapeutic approaches for KS.

To better understand the roles of kinases in KSHV latency, we applied **KiR (Kinome Regularization)** analysis to a poly-pharmacology-based functional screen of the human kinome in iSLK cells latently infected with a KSHV recombinant containing a lytic replication reporter gene. This analysis utilized large-scale drug-target profiling data, regularized regression, and broadly selective chemical tool compounds to **pinpoint specific kinases** and associated networks governing the KSHV latent-to-lytic replication switch and to **identify kinase inhibitors** predicted to regulate KSHV latency maintenance. This kinome screening approach predicted several kinases not previously linked to KSHV latency, as well as others that have been reported in other screens, including EGFR and MAPKs. Additionally, based on this screen, we predict that several FDA-approved kinase inhibitors that are known to restrict the replication of other herpesviruses also regulate the maintenance of KSHV latency. Current studies are underway to validate the predicted kinases and kinase inhibitors in regulating KSHV latency maintenance. Together, these findings will inform on KSHV latency dependency factors and therapeutic design for KS.

Result 1: Successful optimization of a polypharmacological kinome screening system.

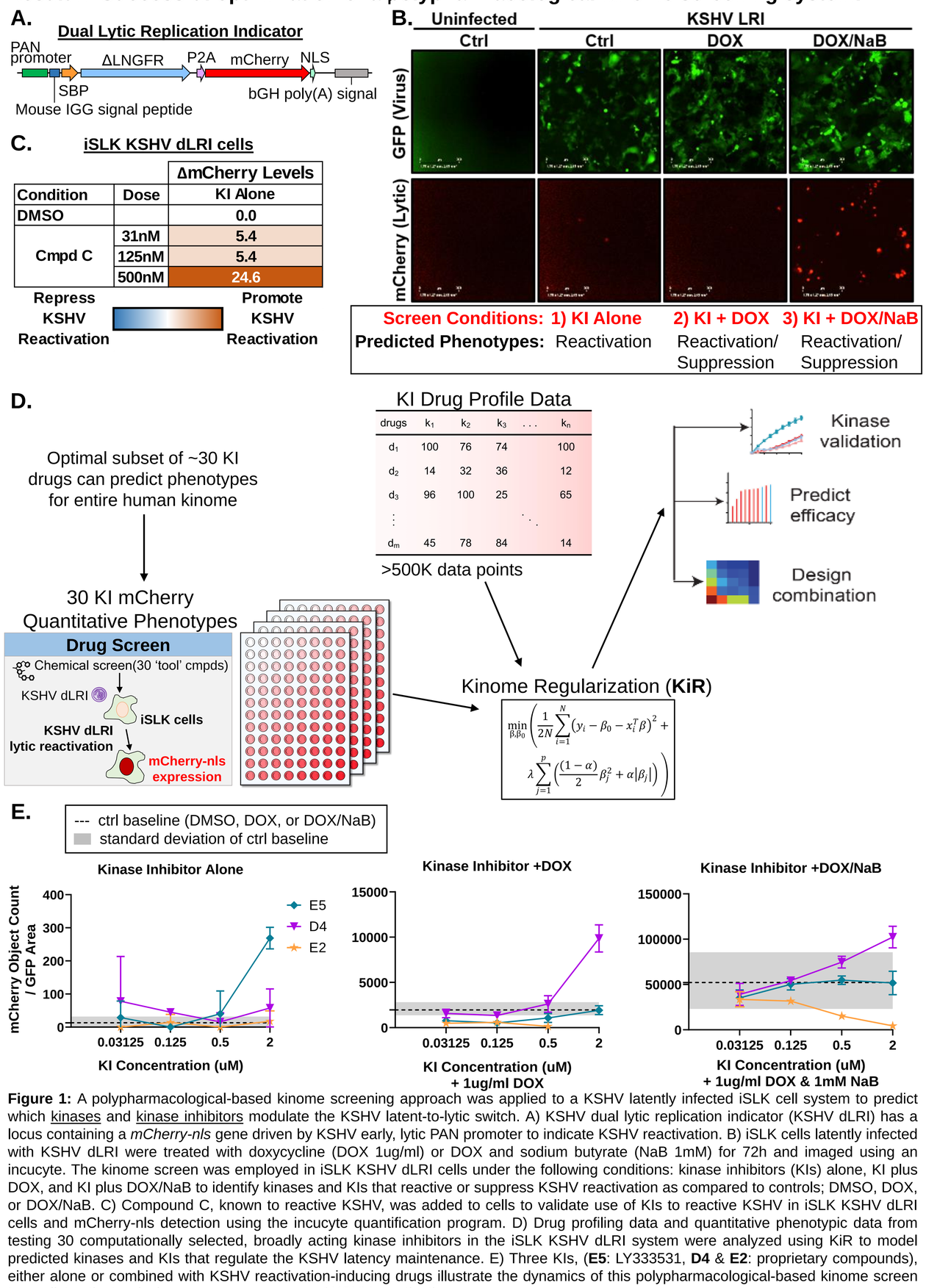


Figure 1: A polypharmacological-based kinome screening approach was applied to a KSHV latently infected iSLK cell system to predict which kinases and kinase inhibitors modulate the KSHV latent-to-lytic switch. A) KSHV dual lytic replication indicator (KSHV dLRI) has a locus containing a *mCherry-nls* gene driven by KSHV early, lytic PAN promoter to indicate KSHV reactivation. B) iSLK cells latently infected with KSHV dLRI were treated with doxycycline (DOX 1ug/ml) or DOX and sodium butyrate (NaB 1mM) for 72h and imaged using an incucyte. The kinome screen was employed in iSLK KSHV dLRI cells under the following conditions: kinase inhibitors (KIs) alone, KI plus DOX, and KI plus DOX/NaB to identify kinases and KIs that reactive or suppress KSHV reactivation as compared to controls; DMSO, DOX, or DOX/NaB. C) Compound C, known to reactive KSHV, was added to cells to validate use of KIs to reactive KSHV in iSLK KSHV dLRI cells and mCherry-nls detection using the incucyte quantification program. D) Drug profiling data and quantitative phenotypic data from testing 30 computationally selected, broadly acting kinase inhibitors in the iSLK KSHV dLRI system were analyzed using KiR to model predicted kinases and KIs that regulate the KSHV latency maintenance. E) Three KIs, (**E5**: LY333531, **D4** & **E2**: proprietary compounds), either alone or combined with KSHV reactivation-inducing drugs illustrate the dynamics of this polypharmacological-based kinome screen to predict activators/enhancers or suppressors of KSHV reactivation.

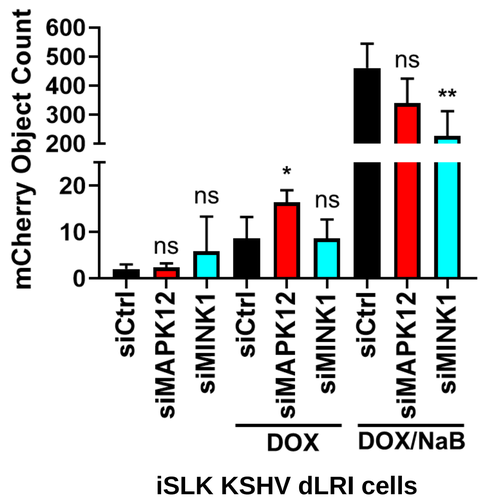
Result 2: MNK1 is predicted to regulate KSHV lytic reactivation in iSLK KSHV dLRI cells.

A. DOX Screen

Kinase	Model #1 Rank	Model #2 Rank
MNK1	19	29
MAPK12	9	26

DOX/NaB Screen

Kinase	Model #1 Rank	Model #2 Rank
MNK1	5	1
MAPK12	152	218



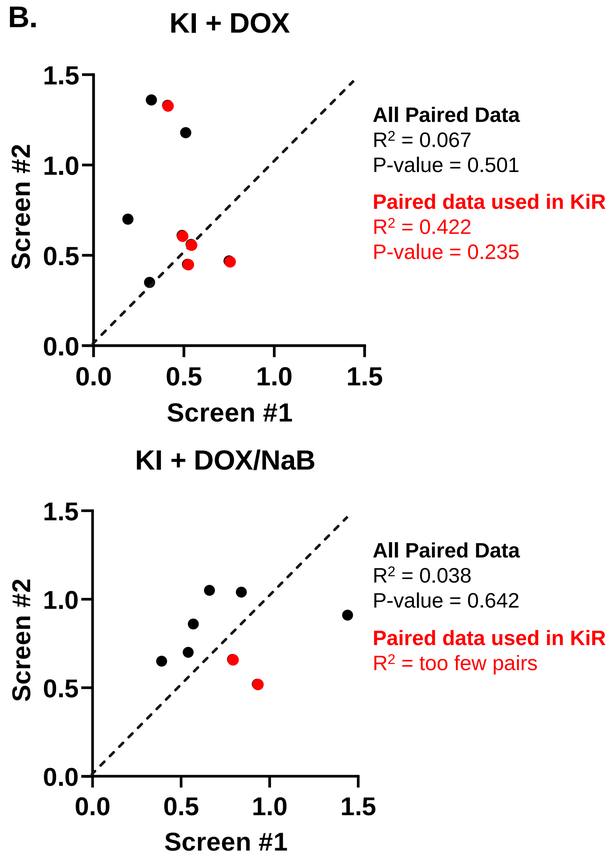


Figure 2: KiR modeling predicted MNK1 and to a lessor degree MAPK12 (ERK6) as potential regulators of the KSHV latent-to-lytic replication switch. A) The kinome screens were conducted in combination with DOX or DOX/NaB. Model #1 represents initial screen analysis. Model #2 includes additional repeats. MNK1 was consistently predicted in Model #1 and Model #2. B) KIs tested in both screen #1 and #2 were graphed to determine screen reproducibility. Exclusion of data (●) with >20% cell death as compared to controls or data with high variability in technical replicates, increased the correlation coefficient comparing screen #1 and #2 data for KI + DOX condition (top graph, ●). C) iSLK KdLRI cells were transfected with 100nM siRNA alone or in combination with DOX or DOX/NaB. 72h post transfection/ treatment with drugs, cells were imaged and mCherry levels were quantified using an incucyte. N=1. P-values: * < 0.05, ** < 0.01; ns = not statistically significant.

Result 3: KiR analysis predicted several kinases and kinase inhibitors as regulators of the KSHV latent-to-lytic replication switch.

A. Model predicted 'informative' kinases

KI + DOX	KI + DOX/NaB
IRAK4	MNK1
MAPK14	ITK
MAP3K8	MNK2
CLK1	EGFR
TEC	PTK5
EGFR	MAP3K8
BRSK2	AURORA A
ARK5	TEC
MET	PKG2
CSNK1D	ERBB4

B. Model predicted kinase inhibitors

KI + DOX		KI + DOX/NaB	
NCGC00344999	0.5μM	Staurosporine	0.5μM
K252a	0.5μM	PF-477736	0.5μM
Staurosporine	0.5μM	AZD3463	0.5μM
JAK3 Inhibitor VI	0.5μM	GSK-650394	0.5μM
Lestaurtinib	0.5μM	AV 412	0.5μM
IRAK1/4 Inhibitor	2.0μM	ASP-3026	0.5μM
NCGC00263020-01	0.5μM	Gefitinib	2.0μM
Go6976	0.5μM	Lestaurtinib	0.5μM
AP26113	0.5μM	Afatinib (BIBW2992)	0.5μM
IRAK inhibitor 5	0.5μM	BMS-794833	0.5μM

Figure 3: KiR modeling in the iSLK KSHV dLRI system for conditions KI plus DOX and KI plus DOX/NaB predicted both previously known regulators of herpesvirus reactivation and uncharacterized kinases and KIs. A) EGFR and MNK1 kinases and the MAPK signaling pathway are published regulators of KSHV latent-to-lytic replication switch. Other predicted kinases have not been tested in KSHV reactivation or latency maintenance. B) Staurosporine and K252a restrict EBV lytic gene expression and Gefitnib and Afatinib respectively enhance and supress KSHV reactivation in cells treated with reactivation inducing drugs. The other predicted kinase inhibitors are uncharacterized with respect to KSHV latent-to-lytic replication modulation. Kinases (●) and KI drugs (●) that will be tested to validate KiR predictions are indicated in the above tables.

Conclusion: The polypharmacological-based kinome screening approach combines drug profiling data with phenotypic data from ~30 computationally selected kinase inhibitors to conduct a network-based analysis of all 518 kinases in the human kinome and predict which kinases and FDA-approved kinase inhibitors regulate a specific phenotype. This kinome screening approach was conducted in KSHV dLRI iSLK cells to predict kinases and kinase inhibitors that positively or negatively regulate KSHV reactivation. First, a recombinant KSHV with a dual lytic replication indicator was generated for accurate quantification of KSHV reactivation using an incucyte to capture nuclear localized mCherry fluorescence data. Second, the kinome screen was conducted under three conditions to predict kinases and kinase inhibitors that either reactivate KSHV or suppress induction of lytic replication by measuring KSHV reactivation for KI alone conditions and each KI tested with KSHV reactivation-inducing drugs. KiR modeling predicted several kinases and KIs as KSHV latency regulators. Functional studies are required to determine the directionality of predicted kinases and KIs. Kinase depletion assays are underway for predicted MAPK12 (ERK6) and MNK1 in the presence of DOX or DOX/NaB, respectively. Validation of kinases and KIs capable of regulating the KSHV latent-to-lytic replication switch will inform our understanding of virus-required host factors and potential therapeutic agents for treatment of KS.

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